

Surface Treatments and Functional Coatings for Biocompatibility Improvement and Bacterial Adhesion Reduction in Dental Implantology

*Original*

Surface Treatments and Functional Coatings for Biocompatibility Improvement and Bacterial Adhesion Reduction in Dental Implantology / Mandracci, Pietro; Mussano, Federico; Rivolo, Paola; Carossa, Stefano. - In: COATINGS. - ISSN 2079-6412. - ELETTRONICO. - 6:1(2016), pp. 1-22. [10.3390/coatings6010007]

*Availability:*

This version is available at: 11583/2655528 since: 2016-11-10T14:52:38Z

*Publisher:*

MDPI

*Published*

DOI:10.3390/coatings6010007

*Terms of use:*

openAccess

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

(Article begins on next page)

Review

# Surface Treatments and Functional Coatings for Biocompatibility Improvement and Bacterial Adhesion Reduction in Dental Implantology

Pietro Mandracci <sup>1,\*</sup>, Federico Mussano <sup>2</sup>, Paola Rivolo <sup>1</sup> and Stefano Carossa <sup>2</sup>

<sup>1</sup> Materials and Microsystems Laboratory (ChiLab), Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi 24, Torino 10129, Italy; paola.rivolo@polito.it

<sup>2</sup> Department of Surgical Sciences, CIR Dental School, Università di Torino, via Nizza 230, Torino 10127, Italy; federico.mussano@unito.it (F.M.), stefano.carossa@unito.it (S.C.)

\* Correspondence: pietro.mandracci@polito.it; Tel.: +39-011-090-7383; Fax: +39-011-090-7399

Academic Editor: James Kit-hon Tsoi

Received: 15 October 2015; Accepted: 20 January 2016; Published: 27 January 2016

**Abstract:** Surface modification of dental implants is a key process in the production of these medical devices, and especially titanium implants used in the dental practice are commonly subjected to surface modification processes before their clinical use. A wide range of treatments, such as sand blasting, acid etching, plasma etching, plasma spray deposition, sputtering deposition and cathodic arc deposition, have been studied over the years in order to improve the performance of dental implants. Improving or accelerating the osseointegration process is usually the main goal of these surface processes, but the improvement of biocompatibility and the prevention of bacterial adhesion are also of considerable importance. In this review, we report on the research of the recent years in the field of surface treatments and coatings deposition for the improvement of dental implants performance, with a main focus on the osseointegration acceleration, the reduction of bacterial adhesion and the improvement of biocompatibility.

**Keywords:** dental implants; titanium; surface modification; surface coatings; plasma polymers; osseointegration; antibacterial; biocompatibility

## 1. Introduction

The goal of modern dentistry is to restore the patient to normal function, speech, health and aesthetics. A dental implant is an artificial tooth root that is surgically placed into jaws so as to hold a replacement tooth (the crown) in place. Modern oral implantology began when Per-Ingvar Brånemark, while conducting experiments *in vivo* using intra-bony titanium chambers, discovered the particular connection this metal was capable of developing within the recipient tissue [1]. The concept of osseointegration, *i.e.*, a stable and direct bone-implant interlock, was originally exposed in two fundamental publications [2,3]. Currently, commercially pure titanium and Ti–Al–V alloy have become the gold standard in implant dentistry, although ceramic materials with the use of zirconium dioxide and innovative metallic alloys are attracting increasing interest in implantology. The main route adopted by research and industry to enhance osseointegration has traditionally entailed roughening techniques [4,5], with good outcomes in terms of bone to implant interlock. Common roughening techniques—usually subtractive—are based on mechanical (grit blasting), chemical (acid or alkaline etch), electrochemical (anodization) and physical methods (plasma spray) [6].

The possible role of roughened surfaces in favoring the development or at least the maintenance of peri-implantitis [7] has, however, recently questioned this approach and prompted the use of different surfaces, such as machined ones. Additive modification methods have, therefore, acquired

increasing interest among the scientific community to reduce possible microbial infection of clinically exposed dental implant surfaces, while preserving the advantages roughness ensures during bone healing. Furthermore, the ability to modulate surface topography in a more controlled way and, to some extent, to alter the surface chemistry independently of the microscale has induced research to new and sophisticated proofs of concepts previously deemed impossible. Multicomponent and multifunctional nanostructures have become relevant in the field of biomedical materials [8]. Indeed, the most promising materials for bone regeneration try to reproduce the complex nanostructure of human tissues.

In this review, we report on the most recent surface modification techniques proposed to improve the properties of Ti dental implants. The work has been organized in two separate sections. The first one has been devoted to recapitulate the most recent surface modification methods, divided into surface treatments, inorganic coatings and organic coatings. The second section outlines the most recent achievements that the different types of surface modification have been demonstrated to produce *in vivo* or *in vitro*.

## 2. Surface Modification Methods

In this section, we will review the main methods currently studied for the modification of dental implant surfaces. The technological processes used to change the physico-chemical properties of materials surfaces can be divided in two main categories, depending on whether a layer of new material is applied on the implant surface or if the surface itself is changed by exposing it to physical or chemical agents, such as sand, gases, plasmas, or wet chemicals. In the former case, the method is usually called coating deposition, while the latter is referred to as surface treatment. The deposition of a thin film substitutes the original surface with a new one, with different physical and chemical characteristics, and an interface is created between the coating and the bulk material. This method has the advantage that a wider range of surface properties can be obtained, since the film surface may show characteristics that differ substantially from the ones of the coated substrate. However, since the thin film is deposited on the original surface, there is always the possibility of its partial or complete detachment. The modification of a surface by means of physical or chemical agents, without the deposition of a coating, allows changing several surface properties, such as roughness, morphology and hydrophilicity; however, the range of effects is more limited compared to the case of coating deposition. One advantage of these methods, compared to the additive ones, is that there is no risk of detachment or delamination, since no material has been added to the surface. In the following, we will review the most important methods of surface modifications and coating materials that have been studied in recent years.

### 2.1. Surface Treatments

A wide range of surface treatments have been tested in order to improve the properties of titanium implant surfaces, including accelerating the osseointegration, reducing the bacterial adhesion and protecting from the possible detachment of nanoparticles or ions. Only a few of these processes are used for the fabrication of commercial implants, while many of them, despite having been subject to intensive research, have not been commercially applied so far. Surface treatments rely on the application of different types of physical or chemical agents on the implant surface; these include the following ones:

- sand, glass or ceramic microspheres accelerated toward the surface (sandblasting);
- exposition to acid or alkali chemicals (wet etching);
- anodization;
- exposition to laser radiation;
- exposition to electron beams; and
- exposition to cold plasmas (plasma etching).

Sandblasting (also called gridblasting) is one of the most commonly used types of surface modification processes, thanks to the simplicity, low cost and easiness of application. Microspheres of diameter in the range 10–540  $\mu\text{m}$  are typically accelerated toward the surface to be treated, using a compressed air or nitrogen blow. Most used grid materials include corundum ( $\text{Al}_2\text{O}_3$ ) [9,10], silicon carbide ( $\text{SiC}$ ) [11], titania ( $\text{TiO}_2$ ) [12], hydroxyapatite (HA) [13], zirconia ( $\text{ZrO}_2$ ) [14], silica ( $\text{SiO}_2$ ) [15] and aluminum powders [15]. The main effect of sandblasting is to change the morphology of the treated surface, substantially increasing its roughness. The value of this parameter depends on several factors, including: the type of grid material used, the dimension of the spheres, the energy and angle when they hit the surface, and the duration of the treatment. Typical values of the  $R_a$  roughness are in the range 0.3–3  $\mu\text{m}$  [15,16] as compared to  $R_a$  values lower than 0.1  $\mu\text{m}$  for polished Ti surfaces [15,16]. A side effect of the sandblasting process is the contamination of the surface by the material released by the microspheres during their interaction with the surface. A recent study [15] has evidenced how different types of grid materials and microsphere dimensions lead to different amounts of contamination on the surface, as well as different efficiencies in removing the contaminants already present on the implant. In particular, blasting with  $\text{Al}_2\text{O}_3$  has been found to effectively remove Si contamination from the machined titanium surface, but led to a Al contamination as high as ~15% when microspheres of 54  $\mu\text{m}$  diameter were used.

Another surface treatment that has been widely tested is the etching of titanium surfaces with wet chemicals, such as  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{HF}$ , or combination of these. The main effect of these acid-etching processes is to modify the implant morphology by producing micro-pits of a few microns diameter on titanium surfaces [16,17]. These processes are commonly applied after sandblasting, and the complete process, usually referred to as sandblasting and large grit acid etching (SLA) [18], is often considered the reference surface treatment to which other ones are compared [12,16–18]. The most common process involves the use of  $\text{Al}_2\text{O}_3$  microspheres of 200–540  $\mu\text{m}$  diameter, followed the etching with a mixture of  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  [16]. Typical result of the SLA process consists in the combination of a macro-roughness, provided by the sandblasting process, and a micro-roughness, provided by the acid-etching process [19].

Anodization is another process widely studied, which consists in a potentiostatic or galvanostatic electrochemical oxidation, usually carried out in strong acids, such as  $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{HNO}_3$  or  $\text{HF}$  [17,20]. As a result of this process, a thick porous layer of titanium oxide is formed on the implant surface, which substitutes the very thin and compact native oxide layer. It is also possible to select, to some extent, the phase of the titanium oxide layer among its amorphous, brookite, rutile and anatase forms [20]. Micro-arc oxidation is a modification of the oxidation process in which the implant is immersed in an electrolyte solution containing dissolved salts and acts as the anode of an electric discharge: much higher potential differences are involved with respect to common anodization processes, giving rise to the formation of a plasma on the metal surface [21].

The treatment of Ti surfaces by electron beams has also been tested [22,23], and has been used mainly as a pretreatment for the deposition of CaP coatings on Ti [23]. E-beam irradiation on Ti has been found to reduce the roughness and to improve the nanohardness of the material [22], allowing for the deposition of smoother CaP layers [23]. Laser treatments have also been studied in order to improve the properties of Ti surfaces [24–27]. The main effect of laser radiation on metals, such as Ti, is to produce a localized melting of the material, as a result of the heating produced by the absorption of the high density radiation. Thanks to the very strong absorption of radiation in the metal, the melting process involves only a very thin metal layer under the surface, which is quickly recrystallized after the radiation beam is moved to another portion of the surface, while a  $\text{TiO}_2$  layer is formed due to the interaction of solidifying metal with air [24]. Several types of lasers have been used for the modification of metals, including ruby, Nd:YAG, argon ion,  $\text{CO}_2$  and excimer lasers [26]; however, Nd:YAG seems to be the most experimented laser for the modification of Ti and its alloys for dental implants [24–27]. Typical values of the process parameters are scanning speeds of 100–300  $\text{mm}\cdot\text{s}^{-1}$ , pulse duration of 10–20 ns and pulse energy in the range 0.2–0.5 mJ [25]. The morphology of laser-treated Ti is usually

characterized by an increased roughness compared to machined Ti surfaces, with typical  $R_a$  values of 0.5–2  $\mu\text{m}$  [26,27].

The application of cold plasmas to the Ti dental implant surface has also been studied in recent years. In fact, plasma treatments show many advantages compared to wet techniques, such as acid-etching, including the absence of chemical residuals on the surface, the avoidance of chemical waste and the reduced safety concerns during manufacturing [28]. Plasma treatments can be divided in vacuum plasma treatments (also called reduced pressure plasma treatments) and atmospheric pressure plasma treatments (APP), depending on whether they are carried out in a vacuum vessel at a pressure lower than the atmospheric one, or at atmospheric pressure. The main advantage of reduced pressure plasma is that a lower power is required to activate a plasma discharge at low pressure, allowing for the use of cheaper and simpler power generators. Moreover, these processes allow a better control of the plasma environment, avoiding contamination by the external atmosphere, and a wider range of process gases is available. APP treatments, however, are of much simpler use, since they do not require the use of complex and expensive vacuum systems. Plasma processes are often applied for cleaning and sterilization of dental implants after other surface treatments have been carried out, but they have also been tested for the acceleration of osseointegration [28–31] and the application of antibacterial properties to implants [28,29,32]. Argon and oxygen are the most used gases for these applications [28–33]. Plasma treatments also include plasma immersion ion implantation (PIII) processes, where plasma is used to produce ions, which are accelerated towards the surface of the treated sample and are implanted into it [33]. Plasma nitriding processes have also been tested [34], where the plasma produces nitrogen radicals that reach the Ti surface and react with it forming a TiN layer.

Some surface treatments have also been proposed in order to incorporate some specific chemical elements on the surface of Ti implants. More specifically, the incorporation of strontium (Sr) on the surface of titanium implants has been proposed in order to accelerate the osseointegration process. Modification of Ti surfaces to incorporate Sr ions has been achieved by means of chemical treatments using a strontium acetate solution [35] as well as by means of micro arc oxidation [36].

## 2.2. Inorganic Functional Coatings

In this section, we will review the main types of inorganic functional coatings that have been studied in recent years to improve osseointegration, to enhance biocompatibility or to reduce bacterial adhesion on Ti implants. Among the most studied coating materials for the enhancement of dental implants osseointegration are calcium phosphate (CaP) based alloys [37,38], including hydroxyapatite (HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) [37] and calcium phosphate cements (CPC) [38]. HA coatings can be deposited on dental implants by a variety of techniques, including radio-frequency magnetron sputtering, plasma spray (PS), pulsed laser deposition (PLD) and ion beam assisted deposition (IBAD) [37]. Magnetron sputtering and IBAD techniques are more suitable to obtain compact films with a smooth surface, and are usually able to reproduce quite well the morphology of the implant surface if this was modified before the deposition, e.g., by a SLA process. However, the deposition is slow and the film thickness is usually limited to a few micrometers [37]. Coatings deposited by PLD or PS are characterized by their own roughness, and will result in a rough surface even if the surface of the coated implant was originally very smooth. PS coatings typically show a low density and a high porosity, while PLD coatings are denser, with the surface roughness mainly due to the presence of micrometric droplets on the surface [37]. Calcium phosphate cements are usually formed by the reaction between calcium and phosphate powders in an aqueous phase, leading to the precipitation of CaP [38]. When in contact with the body fluids, these materials lead to the formation of HA nanocrystals. Several methods have been tested for the deposition of CaP coating on Ti implants, including PS, sputtering, sol-gel deposition, and electrophoretic deposition processes, but PS is considered the most successful so far [28]. Strontium-containing HA coatings, grown by PLD or sol gel methods, have also been proposed in order to improve the osseointegration process [39,40].

Titanium oxide ( $\text{TiO}_2$ ) has also been tested as a functional coating for the improvement of Ti implants performance. While the surface of Ti is naturally covered by a thin native oxide layer that is readily formed as the metal surface is exposed to air, its very low thickness prevents the activation of important biological effects. Moreover, its structure is usually amorphous, while some of the most important biological effects, such as antibacterial properties, have been observed especially on crystalline  $\text{TiO}_2$  [20]. The deposition of  $\text{TiO}_2$  thin-films on dental implants is a way to improve the surface biological properties by controlling the structure, morphology and thickness of the  $\text{TiO}_2$  layer exposed to the peri-implant tissues [41–45]. The deposition of  $\text{TiO}_2$  coatings with the incorporation of Ag nanoparticles has also been tested [45], in order to imbue dental implants with antibacterial properties. Several techniques, including magnetron sputtering [41,45], low pressure chemical vapor deposition (LPCVD) [42], metal-organic chemical vapor deposition (MOCVD) [43] and plasma spray [44] were used to achieve the deposition of the titanium oxide for improving osseointegration [42,44] or prevent bacterial adhesion [41,45]. Other oxides, such as  $\text{ZrO}_2$  [41],  $\text{SiO}_x$  [46–49] and  $\text{ZnO}$  [50], have also been tested as alternatives to  $\text{TiO}_2$  to improve the properties of dental implants, reduce the bacterial adhesion [41,46,49,50], improve the biocompatibility [47] or protect from the corrosion exerted by the body fluids [48]. In addition, Zn-doped  $\text{TiO}_2$  has been tested as a possible candidate for bacterial adhesion reduction [50]. These oxides are usually grown by sputtering [41,48], plasma enhanced chemical vapor deposition (PECVD) [46,47,49] or cathodic arc deposition [50], since these techniques allow the deposition of compact layers and allow a good coating adhesion even at low temperature.

Nitride coatings have also been tested as candidates to improve dental implants properties, including TiN [51,52], AlN [48], TaN [53] and Cu- or Ag-doped TiN and TaN [54,55] grown by magnetron sputtering [52,53,55], possibly combined with plasma nitriding [54]. These coatings have been intended mainly to improve the osseointegration [51] the protection against corrosion [48,52–54], and to reduce bacterial adhesion [51,53–55]. In addition, ZrN and ZrCN coatings grown by cathodic arc deposition [56] were tested against their antibacterial properties.

Moreover, Sr-doped brushite coatings grown by electrochemical deposition methods [57] have also been proposed recently as a titanium surface treatment for the acceleration of osseointegration.

### 2.3. Organic Coatings

Various types of organic coatings, polymers (e.g., obtained by plasma discharge) and biomimetic and bioinspired films (e.g., components of the natural cell surroundings) have been explored on biomaterials used for dental implants and orthopedic joint prostheses in order to: get an intimate bone–implant contact, promote osseointegration by proliferation of osteoblasts, prevent for bacterial adhesion or to impart a bactericidal effect to the implant surface.

In this section, a focus will be reported on plasma polymers both used for the direct contact with cells involved in osteogenic process and applied as intermediated layers for the immobilization of several organic species, all enhancing bone repair and osseointegration: Cell Adhesion Molecules (CAMs), cell stimulating molecules and extracellular matrix proteins (ECMs) (e.g., glycans, Poly-aminoacids, collagens, pectin, fibrinogen, laminin, osteopontin, insulin, cytokines, fatty acids, sugars, *etc.*).

Plasma polymer deposition is a well suited technique for the preparation of a defined interface thin layer, e.g., at cell culture plates or at bone-implants, for the covalent or electrostatic immobilization of biologically active molecules aimed to improve the osseointegration (for instance, through an enhanced bone-titanium interface and better soft tissue integration). In the last years, particular attention was devoted to the effect of biochemistry occurring at the interface on initial cellular adhesion and differentiation.

In plasma polymerization, the transformation of low-molecular-weight molecules (monomers) into high-molecular-weight molecules (polymers) occurs with the assistance of energetic plasma species such as electrons, ions and radicals. In many cases, polymers obtained by plasma polymerization



have different chemical compositions as well as chemical and physical properties from those formed by conventional liquid phase polymerization, even if the same monomers are used. This uniqueness results from the reaction mechanism of the polymer-forming process.

Bioactive plasma polymeric films have to fulfill essential requirements, which are important for a subsequent use such as homogeneity, film stability, and adequate type and densities of functional groups or surface charge. They should be adjustable by the choice of adapted plasma process conditions. Different precursors and plasma excitation methods, process parameters and reactor geometries influence the plasma polymerization process and lead to a diversity of plasma-chemical reactions and thus to different film properties [58]. The low pressure plasma processes are the most widely used: microwave (2.45 GHz) [59] or capacitively coupled radio frequency (13.56 MHz) [59–65] activated [60], in planar parallel electrode plates geometry [63–65], in steel chamber [59,63–66] or glass tubes [62,67,68] reactors, applying a continuous [61,67] or pulsed plasma discharge [59,63–65,69].

In particular, the most exploited functional groups in bioactive plasma polymers are:

- $-\text{NH}_2/-\text{CONH}_2$  (amino/amide groups) [59–62,66,69–72];
- $-\text{COOH}/-\text{CHO}$  (carboxylic/aldehydic groups) [63–67,69,73,74]; and
- $-\text{OH}$  (alcoholic groups) [66,68,72,75].

The plasma polymerization of amine-based monomers is an efficient way to prepare bioactive thin film polymers terminated with nitrogen containing functional groups. These surfaces are characterized by a positive surface charge in aqueous environments and thus prone to interaction, especially with hyaluronan, a negatively charged substance of the extracellular matrix, playing a key role in the initial adhesion of osteoblasts to artificial surfaces.

If the precursor contains in the chemical structure  $\text{C}=\text{C}$  double bonds, the combination of plasma and conventional free-radical polymerization is enhanced. For  $\text{C}-\text{C}$  single bonds containing molecules, the only polymerization mechanism possible is the plasma activation. Finke *et al.* started from [59] allylamine (AAM) and ethylenediamine (EDA) to get  $\text{NH}_2$  terminated Plasma Polyallylamine (PPAAm) and Plasma Polyethylenediamine (PPEDA) on polished titanium alloy substrates (Ti-6Al-4V\_P discs) by microwave or radio frequency activated plasma discharges, respectively. In the first case, a microwave (2.45 GHz) plasma reactor was used. After a cleaning/etching step of a continuous wave (cw)  $\text{O}_2$  plasma, the polymerization of AAM occurred on the surface of the substrates, placed in a downstream position, through the application of a MW excited, pulsed, plasma discharge. In the second case, the deposition was carried out in a stainless steel grounded chamber equipped with planar electrodes, using a pulsed RF-generator (13.56 MHz). The RF-electrode was temperature-controlled ( $10\text{--}15\text{ }^\circ\text{C}$ ) to ensure constant temperature of the samples and to set the growth rate of polymerized EDA to  $0.4\text{ nm/s}$  at steady state plasma conditions.

After 1:1 derivatization of  $\text{R}-\text{NH}_2$  by 4-trifluoromethyl-benzaldehyde (TFBA), from the XPS elemental ratio F/C, the resulting PPAAm is characterized by an amino groups density lower than PPEDA, even if the aging degradation is lower for the first.

A third coating, obtained by 13.56 MHz RF magnetron sputtering of nylon 6.6 under  $\text{N}_2/\text{H}_2$  1:1 gas mixture (PSN), was compared to the previous ones. Even if PSN shows a higher density of amines, FTIR characterization revealed a high content of nitriles and amide species and a structure that is not dominated by a hydrocarbon network. Notwithstanding the differences in chemical features, PPAAm, PPEDA and PSN coatings suffer an oxidation effect due to air aging, resulting in the conversion of primary amine to acid amides.

The PPEDA coating for corundum blasted titanium alloy (Ti-6Al-4V) applied in orthopedic surgery [60] can be also obtained by low pressure capacitively coupled radio-frequency (RF) plasma at 13.56 MHz, in the same conditions described in [59], where the processing gas is a mixture of the carrier gas argon and the vapors of the liquid EDA precursor stored in a vessel, held at a temperature of about  $30\text{ }^\circ\text{C}$  to ensure a constant vapor pressure. Immediately after the deposition, the coated samples were stored under ambient conditions in plastic boxes and subjected to an additional sterilization by

$\gamma$ -irradiation. Subsequently, the thin films aging and its influence on the cell adhesion were tested on independent samples after different storage times up to 360 days.

The film aging was in-depth studied by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy by monitoring the evolution *versus* aging time of specific vibrational absorption peaks: stretching modes of C=N and C $\equiv$ N double and triple bonds, C $\equiv$ C triple bonds, C=O and O–H bonds. The post-deposition oxidation under ambient air storage is a very quick reaction. About 60% of the primary amino groups in PPEDA are converted into acid amides and (partially) imides in the first 30 days of storage on air [59]. Finally, acid amides can be hydrolyzed to carboxylic acid groups and ammonia. In addition, nitrile hydrolyzes to the same end products.

The N-rich plasma coatings such as PPAAm [61] were deposited, on anodically oxidized Ti surfaces, also to immobilize hyaluronic acid (HyA), a glycosaminoglycan (ECM substance) that plays an important role in adhesion, migration and proliferation of osteoblast-like cell (MC3T3-E1 ATCC CRL-2593, a clonal pre-osteoblastic cell line derived from newborn mouse calvaria). HyA is negatively charged due to the carboxyl group of glucuronic acid. Therefore, it can interact, on one side, with the aminated and positively charged ( $-\text{NH}_2$  protonation occurs in aqueous solutions at physiological pH) PPAAm surface, and, on the other side, it can boost the first contact of the cell to the surface until the cell synthesizes matrix proteins to mediate the fundamental integrin contact in focal adhesion migration and proliferation. PPAAm was obtained by a RF generated plasma discharge, first pretreating the surface in  $\text{O}_2$ , and then, in presence of the AA vapors. The formed  $\text{NH}_2$ -terminated films was made to react with  $-\text{COOH}$  termination of HyA by a crosslinking activating agent EDC (1-ethyl-3-(3-dimethylamino) propyl carbodiimide)-NHS (N-Hydroxysuccinimide) in aqueous solution, in order to form a covalent amide based bond, which presence was confirmed by ATR-FTIR characterization (weak peak near  $1700\text{ cm}^{-1}$  assigned to the carbonyl in amide group).

The surface undergoes (observed by SEM and AFM) significant changes as a result of anodic oxidation, plasma polymerization and subsequent HyA immobilization (Scheme 1 in [61]). The surface roughness of anodized Ti/PPAAm/HyA sample increased to give a characteristic hill–valley structure on the  $10\text{ }\mu\text{m}$  scale so promoting cell adhesion.

In the frame of biomimetic surface modifications, PPAAm coating, deposited on titanium was recently studied with respect to the covalent immobilization of extracellular matrix (ECM) proteins, such as fibrinogen, collagen, laminin, osteopontin [62] and pectins, which are plant-derived polysaccharides [70]. The cellular response elicited in human umbilical cord cells (HUVECS) [62] and human osteoblasts (HOBS) [62,70], was evaluated. Cell adhesion can be influenced selectively according to the surface modifying ECM protein.

In [62], the deposition of PPAAm occurred in a custom-made plasma reactor. The basic setup consists of a cylindrical Pyrex glass tube. The alternating current with radio frequency (13.56 MHz) was applied over an electrode coiled around the glass tube. The vacuum inside the chamber was generated by a vacuum pump. To protect it from effluent monomer contamination, a cold trap, cooled with liquid nitrogen was used. Before admission through the needle valve, allylamine was degassed at least three times using liquid nitrogen to remove dissolved gas contaminations. To produce reproducible results, plasma polymer films of a thickness of approximately 160 nm were used. The amino terminated surface, in this case, were crosslinked to the proteins of interest (fibronectin, collagen, osteopontin, and laminin) by means of a PBS solution of, *x*-bis-N-hydroxysuccinimide polyethylene glycol (Di-NHS linker), as shown in Figure 1 in [62]. After the surface activation by the Di-NHS linker, the proteins were immobilized by placing the samples with the upside on a 30- $\mu\text{L}$  droplet of each solution for 1 h. After rinsing with PBS, samples were carefully dried under an air stream. All the process flow was characterized in terms of kinetics of adsorption by SPR measurements, using, as deposition substrates for PPAAm, gold slides. Low adsorption efficiency was observed only for osteopontin. One possible explanation is the low concentration of the solution used for the osteopontin immobilization and its small size, in the range between 44 and 75 kDa, which produces a low perturbation of SPR signal, in terms of refractive index change.



Pectin Rhamnogalacturonan-Is (RG-Is), isolated from potato and apple [70], was covalently immobilized on the PPAAm coating deposited on Ti discs, placed on a water cooled grounded electrode of an RF (13.56 MHz) plasma reactor, according to the following process parameters: a deposition time of 30 s with a discharge power of 100 W and pulsed plasma with a duty cycle of 4 ms on and 4 ms off. The RG-Is was covalently coupled via reaction between its carboxyl groups and the primary amino groups on the surface of the aminated Ti discs. EDC/sulpho-NHS was used as coupling agent. If RG-Is contains a high amount of galactose, it is able to increase mineralized matrix formation of osteoblastic cells *in vitro*.

Schröder and Finke [69] also explored the plasma polymers obtained by acrylic acid (PPAAc) in comparison with PPAAm, coming from allylamine, in terms of capability to influence the adhesion of human mesenchymal stem cells (hMSCs) and inflammation processes on titanium substrates, which are representative of real implant surfaces. While carboxyls (from PPAAc) lead to negative surface charges, amines (from PPAAm) are strongly basic at biological pH of 7.5, leading to positive surface charges. The effect of the negatively and positively charged chemical functional groups introduced by plasma polymerization on the adhering cells was studied.

PPAAc is often obtained by RF plasmas with a high density of carboxylic groups. The setting of discharge parameters is crucial in order to get a film stable and insoluble in water. This goal could be achieved through a high degree of cross-linking, but the consequent chains high fragmentation at high discharge power determinates a strong decrease of  $\text{-COOH}$  groups density. Notwithstanding, it was demonstrated that a finely tuned pulsed RF plasma discharge allows obtaining a high surface density of carboxylic groups ( $10^{15}\text{--}10^{16}$   $\text{-COOH}/\text{cm}^2$ ), stable in aqueous solutions, able to covalently graft  $\text{NH}_2$  terminated c-DNA probes, complementary to *Listeria monocytogenes* oligonucleotides, for microarray diagnostics [63] and proteins (Protein A) if deposited on porous silicon matrices [64]. Moreover, when applied to polypropylene hernia-repair meshes [65], PPAAc can be successfully loaded with silver nanoparticles that were shown to be moderately released in physiological media. Two different actions can so be exploited: (i) immediately after the surgical operation, the silver released in solution can exert the antimicrobial action in the aqueous environment nearby the surgical site; and (ii) the silver, remained at the biomedical device surface, can reduce the formation and/or the adhesion of bacteria biofilms onto the prostheses interface.

In [69], PPAAc was deposited on polished Ti discs (TiP) by applying a microwave activated plasma discharge (reactor described in [59]), and using pulsed discharges with different “plasma on” times and duty cycles (DC). A liquid handling system allowed exact dosing of the precursors, by a calibrated needle valve. The films, whose thickness was about 50 nm, characterized by X-ray photoelectron spectroscopy (XPS) and ATR-FTIR, showed high content of carboxylic groups. PPAAm was also deposited on TiP by the same apparatus with a different DC. The comparison in terms of stability of the different chemical groups (percentages of bonding components in C1s peak, by XPS), shown in Figure 2 in [69], after 10 min sonication in water, confirmed the reliability of the films in aqueous media. The Z-potential characterization was able to widely discriminate the opposite charged surface of the two kind of coatings.

Plasma co-polymerization [67] of a mixture of acrylic acid and ethylene allowed to combine in the resulting PPethAAc film the advantages of each precursor, the first characterized by a functional group, such as acrylic acid ( $\text{-COOH}$ ), and the second acting as a chain extender or cross-linker.

The films were grown in RF (13.56 MHz) glow discharges in a tubular glass plasma reactor equipped with two parallel internal and symmetric stainless steel electrode plates. The upper electrode, shielded, is connected to a RF generator through a matching network; the lower, substrate holder, is grounded. Argon was used as carrier gas for acrylic acid vapors and ethylene gas and to sustain the plasma discharge. Different experimental conditions were used to tune the surface density/distribution of functional carboxyl groups and to obtain substrate-adherent coatings, stable in water. The presence of ethylene goes to influence this last goal. The films were deposited on c-Si for the XPS characterization and water stability tests. PTFE disk were used as substrates for the estimation of the density of  $\text{-COOH}$

group by Toluidine Blue-O (TBO) colorimetric titration before and after water soaking. Preliminary biological *in vitro* experiments in Polystyrene (PS) Petri dishes coated with a PPethAAc film were used as substrates for a preliminary cell culture experiment with Saos-2 osteoblast cell lines. The best quality film was obtained by a fixed ratio of  $\Phi_{\text{Eth}}/\Phi_{\text{AA}} = 3$ , with RF power values of 30, 50 and 80 W. In particular, the 30 W-obtained coating shows a highly good stability, after immersion in water of the exposed  $-\text{COOH}$  groups. This result would have been very hard to reach if only AA had been used as feed, at low RF power. Ethylene does not contribute to the coatings with oxygen containing groups but introduces cross-links in the network, thus discouraging interactions between PPethAAc and water and reducing film solubility. TBO data show that the density of  $-\text{COOH}$  groups in PPethAAc coatings decreases when the RF power is increased ( $7 \times 10^{-6}$  mol/cm<sup>2</sup> after water soaking). Concerning cell adhesion, osteoblasts grew on 30 W PPethAAc surfaces even better with respect to the bare Petri control, as proved by the higher number of adhering cells.

PPAAc was successfully deposited [73] on the surface of biodegradable polycaprolactone (PCL) scaffolds in order to immobilize the recombinant human bone formation protein-2 (rhBMP-2), a class of locally signaling molecules that promote bone formation by both osteoconduction and osteoinduction, so producing a substrate that can replace bone tissue for a long period owing to the low biodegradation rate. The plasma process was carried out by using a capacitively coupled RF plasma polymerization system [66], consisting in a vertically placed stainless steel cylindrical chamber. The RF electrode is placed horizontally at the center of the chamber and connected to an RF (13.56 MHz) source and the ground steel electrode holds the substrate to be processed. An oxygen plasma pretreatment was conducted to decontaminate and activate the sample surface. Acrylic acid monomer was evaporated at room temperature (32 °C in a water bath) from a container and its vapor was introduced into the vacuum chamber. In addition, in this case, the quantification of the  $-\text{COOH}$  surface density was done by TBO colorimetric titration (on 2 cm diameter titanium discs that are inert towards the chemical procedure), evidencing that the density of carboxyl groups decreased with increasing plasma discharge power. At a plasma discharge power of 60 W, the density of carboxyl groups was 0.04 mmol/cm<sup>2</sup>, ensuring a moderate wettability that promotes the adsorption of an appropriate amount of proteins and thus the adhesion of cells. Moreover, the obtained 85 nm coating is quite stable in water. Finally, after incubation of rhBMP-2 proteins, the ATR-FTIR characterization showed that the peaks of the bonds related to the amide moieties of the protein is present, so confirming that rhBMP-2 is adsorbed on the functionalized PCL scaffolds.

In addition to Poly  $\epsilon$ -caprolactone (PCL), poly D,L-lactic acid (P<sub>DDL</sub>LLA) scaffolds, with controlled pore dimensions (100–200  $\mu\text{m}$ ), were used as substrates of deposition of plasma polymers. In particular [71], low pressure RF (13.56 MHz) Glow Discharges were applied in presence of ethylene/N<sub>2</sub> mixtures (for PCL) and allylamine (for P<sub>DDL</sub>LLA) to get N-containing species penetration inside the core regions of scaffolds. The obtained cell adhesive coatings are shown to increase the affinity of Saos2 osteoblast and 3T3 fibroblast cell lines, respectively.

PCL scaffolds were modified in a C<sub>2</sub>H<sub>4</sub>/N<sub>2</sub> mixture (1:3), in continuous mode (a stainless steel parallel plate plasma reactor was used), obtaining a N-containing plasma polymerized ethylene (PPE:N) film, whose thickness on flat PCL substrates was 500 nm. Then the scaffolds were plasma treated in H<sub>2</sub> in order to impart a different surface chemical composition to the scaffold periphery e.g., rich in  $-\text{NH}_2$  groups (short H<sub>2</sub> plasma treatment time) or rich in hydrocarbon moieties (long H<sub>2</sub> plasma treatment time). P<sub>DDL</sub>LLA scaffolds (a glass reactor, equipped with two external, capacitively coupled electrodes, was used) were first cleaned with an O<sub>2</sub> plasma etching and then coated in pulsing power mode, with a plasma-polymerized allylamine coating (PPAAm), whose thickness on flat P<sub>DDL</sub>LLA surfaces was 100 nm. A further film, 20 nm thick on flat surfaces, was then plasma deposited in continuous power mode from hexane vapors, thus obtaining a plasma polymerized hexane (PPHex film).

This new double deposition process from allyl amine (core) and hexane (periphery), created a chemical gradient from the top to the bottom of the scaffolds, that increased the metabolic activity of cells inside the scaffolds. Therefore, by homogeneously decorating the scaffolds with a cell-adhesive

PPAAm film and a cell-repulsive thin PPHex barrier only on the outer surface, cells are forced to faster colonize the core of the scaffold.

Some authors [66,72] compared the PPAAm and PPAAc coating with the one obtained starting from allylalcohol (PPAAl) on 2 different kind of substrates: SLA-treated titanium (Ti) surfaces [72] and magnesium (Mg) discs [66], as screws and plates made of Mg alloys provide stable implant materials that degraded *in vivo*, eliminating the need for a second operation and promoting osseointegration with an increased bone mass around the Mg alloys. In both cases, a capacitively coupled radio frequency (13.56 MHz) discharge plasma device was used for films deposition. Beyond all described low pressure plasma technique, [74] non equilibrium atmospheric pressure plasma processes, in particular based on dielectric barrier discharges (DBDs) mechanism, can be applied in the frame of deposition of plasma polymers on biomaterials. Starting from the aerosol of the organic precursor, the plasma system reported in Figure 1 in [74] was used to deposit –CHO containing coatings from lactic acid (PPLA) and tetraethylene glycol dimethyl ether (PEO-like) of possible interest in biomedical applications as biodegradable and non-fouling polymers, respectively. For the first coating, a water DL lactic acid (LA) solution 90% v/v was atomized with N<sub>2</sub> or He by using a constant output atomizer. The DBD deposition system includes two parallel plate electrodes each covered with a 3 mm dielectric glass plate. N<sub>2</sub> or He were used as carrier gas, the LA aerosol was generated by means of a constant flow of N<sub>2</sub> or He. The LA aerosol mixed with N<sub>2</sub> or He carrier was fed at one-end of the inter-electrode gap (tunable according the process requirements). Deposition time (10 min) and applied frequency (4 kHz) were kept constant. For the second coating, liquid tetraethylene glycol dimethyl ether (TEGDME) was atomized with He and admitted longitudinally the two square parallel plate electrodes located in a Plexiglas enclosure. Each electrode was covered with a 1.9 mm thick alumina dielectric plate. The TEGDME aerosol was let in the discharge with a constant He flow rate. From ATR-FTIR characterization, it results that the PPLA film retains the ester groups, important for applications where the (bio)degradation of the material is required, since these groups are directly involved in the degradation reactions. These optimal condition are obtained when He is used as buffer gas instead of N<sub>2</sub>. For PEO-like coatings, a balance between a high percentage of CH<sub>2</sub>CH<sub>2</sub>O– ether groups and a slight cross-linking has to be achieved in the coating to obtain films with non-fouling properties and good stability in water at the same time. A crucial parameter seems to be the chemical composition of the feed rather than the applied power. Thus, decreasing the flow rate at fixed applied Voltage, was found by XPS analysis a PEO (high density of the CH<sub>2</sub>CH<sub>2</sub>–O ether functionalities) character of 70%, comparable to those deposited at low pressure.

Among antibacterial applications of plasma polymers, a recent and promising study on terpinen-4-ol [68] was conducted by using a custom-manufactured glass deposition chamber equipped with copper electrodes capacitively coupled to the reactor so that RF (13.56 MHz) energy is delivered into the deposition chamber [75]. The resulting plasma polymerized polyterpenol (PPT) films are able to retard adhesion and proliferation of several human pathogenic bacteria thanks to the exposed O-containing functional groups that can be tuned by changing the RF power. Improved stability was observed for films fabricated at higher RF powers due to the increased degree of cross linking and the decrease in the oxygen content associated with the higher deposition power; the decrease in oxygen containing functional groups was responsible for the increased hydrophobicity of the polymer surface, so enhancing its anti-fouling properties.

### 3. Biological Effects

The present section focuses on the biological effects exerted by surface modifications, especially additive techniques, suitable for bony interfaces with a particular interest for both organic and inorganic thin film coatings. For the sake of clarity, a short outline of the most recent research achievements will be portrayed by subdividing the matter into:

- bioactive surface modifications (*i.e.*, releasing signal molecules, ions or drugs), for enhancing bone quality or with antimicrobial features;

- structural surface modifications (*i.e.*, treatments aiming at reproducing either peculiar features of the recipient tissue or artificial topographies capable to elicit a biological response), such as Hydroxyapatite (HA) coatings and nanostructured surface modifications.

### 3.1. Bioactive Surface Modifications

#### 3.1.1. Enhancing Bone Quality

Strontium (Sr) has been described as a possible therapy to promote bone growth in osteoporotic patients, as it both increases new bone formation and reduces bone resorption [76]. Interesting attempts have recently been proposed to functionalize titanium implants with strontium. Micro-arc oxidation, alkali treatment and ion exchange were used to incorporate Sr [36] so as to attain a Sr-doped Ti surface. The modified surface remarkably enhanced the adhesion, spreading and osteogenic differentiation of mesenchymal stem cells *in vitro*, even when compared to a control chemically and topographically identical except for Sr content. Sr-doped Ti proved its increased bioactivity in the ovariectomized rat femur model *in vivo*, promoting a significant osseointegration.

With a partially similar approach, a hierarchical hybrid micro/nanorough strontium-loaded titanium surface was fabricated through hydrofluoric acid etching followed by magnetron sputtering [77]. This surface modification significantly improved the proliferation and differentiation of osteoblasts compared with a smooth titanium surface, a micro-roughened titanium surface treated with hydrofluoric acid etching, and a strontium-loaded nano-roughened titanium, which was confirmed also by the bone to implant contact obtained at 12 weeks in rats and the pullout tests performed. These data suggest that the enriching titanium surfaces with strontium may be a viable option for clinical use.

Similar effects could be induced by bisphosphonates as reported by Bosco *et al.* [78] who functionalized hydroxyapatite nanocrystals with alendronate and demonstrated the inhibitory effects of the coating on osteoclast-like cells. To conjugate the useful effects of strontium and zoledronate (ZOL) Combinatorial Matrix-Assisted Pulsed Laser Evaporation (C-MAPLE) was applied to deposit gradient thin films with variable compositions of Sr-substituted hydroxyapatite and zoledronate modified hydroxyapatite on titanium substrates [79]. The peculiar composition of the thin films promoted bone growth and inhibited bone resorption, with a promising synergistic effect in cell cocultures of osteoblasts and osteoclasts.

Layer by layer apposition has been conveniently described as a possible way to attain coatings capable of dismissing a range of bioactive molecules. It is the case of Bone morphogenetic protein 2, a well-known bone inducer introduced into microporous architectures mimicking the extracellular matrix [80]. In this study, self-assembling polydopamine (PDA) microcapsules, optimal for growth factor immobilization and release, were coated with polystyrene (PS) microspheres and assembled using positively charged chitosan (CHI) layers. The produced microporous PDA architectures allowed the sustained release of BMP-2.

A layer-by-layer adsorption of polyelectrolytes albumin and heparin was proposed for controlled binding of basic fibroblast growth factor (FGF-2) [81]. Interestingly, the bioactivity of the adsorbed FGF-2, as assessed *in vitro*, was more pronounced on surfaces at a lower surface concentration of the growth factor. Finally, to achieve an even finer control over the peri-implant bone a sophisticated technology has been proposed based on siRNA nanoparticles embedded in chitosan grown recurring to the layer by layer technique [82].

#### 3.1.2. Antimicrobial Features

Titania nanotubes engineered by electrochemical anodization process have been proposed to develop drug-releasing implants in orthopedics and dentistry [83–85] owing to their biocompatibility (for a complete review, please refer to [83]). As the prolongation of drug release from these nano-reservoirs is still a challenge, the relation between nanotube dimensions (diameter, length, aspect ratio and volume) and drug release rate was investigated finding that systems with higher

aspect ratios released drug slower [84]. Titanium nanotubes could become a feasible technology for real-life clinical applications. This will imply the implementation of different approaches to overcome some technical and commercial challenges.

Silver is traditionally a well-known and widely used antibacterial agent. Codoping dental implants with silver by femtosecond laser doping was proposed, obtaining good results [86]. Likewise, Silver-sourced plasma immersion ion implantation (Ag-PIII) was used to modify the surface of stainless steel [87]. In a clever approach, thin film coatings containing a binary Ag-Ti gradient were deposited generating a gradient of the bioactive ion [88]. It is noteworthy that the structural properties, such as varied porosity and degree of crystallinity, rather than the amount of incorporated Ag, governed the Ag<sup>+</sup> release. The coating also demonstrated *in vitro* apatite-forming abilities, where structural variety along the sample was shown to alter the hydrophilic behavior, with the degree of hydroxyapatite deposition varying accordingly.

Among the numberless techniques already reviewed in the scientific literature concerning drug releasing systems for preventing the bacterial colonization of intrabony prostheses, the authors wish to quote two recent studies. Using as a carrier a novel lipid-based material that may be applied as an implant coating at point of care, antibiotics such as amikacin and vancomycin were loaded and tested against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* [89]. Very promisingly, antibiotic-loaded coatings reduced attachment of bacteria *in vivo*, although future evaluations are needed to include preclinical models to confirm therapeutic efficacy. A versatile method with tailored drug release profile has been described by Chen *et al.* [90]. Briefly, an electrophoretic deposition technique was developed for the fabrication of antibiotic-loaded PHBV microsphere (MS)-alginate antibacterial coatings. From the qualitative antibacterial tests, MS containing coatings showed excellent inhibition effects against *Escherichia coli* (*E. coli*), suggesting that the proposed technique may be considered a promising approach for the controlled *in situ* delivery of drug or other biomolecules.

### 3.2. Structural Surface Modifications

#### 3.2.1. The Inorganic Bone Matrix: Hydroxyapatite

Calcium phosphate ceramics, such as hydroxyapatite (HA), have been traditionally the first choice when bone matrix is to be reproduced because of their good biocompatibility and bioactivity [91]. The growing awareness of the major role played by the physico-chemical features of surfaces in ruling the fate of a biomaterial/host tissue interaction, stimulated an intense research activity aimed at finding preparation/modification procedures that would result in HA particles exposing surfaces with optimized properties [92–94]. Relevant advances in the elucidation of HA surface structure-properties relationships were obtained for micrometric hydroxyapatite particles terminated by defined crystal planes (calcium hydroxyapatite belongs to the P63/m space group), that exhibited two families of different binding sites, called C and P sites, respectively [95]. C sites, should act as adsorbing sites for acidic proteins, whereas P sites are expected to behave as adsorbing sites for basic proteins. Evolution in the preparation techniques has led to methods for obtaining HA in nanosized forms, mimicking those naturally occurring in bone [96]. Interestingly, nano-HA exhibited improved *in vitro* performances in tests with osteoblasts [97,98]. Structure is also important for biomimetism, as the mineral component of bone has well defined features in relation to bone tissue function and age [99,100]. In this respect, the possibility to turn quite smoothly the bulk structure from highly to poorly crystalline or actually disordered by changing preparation conditions, such as temperature, presence of anionic and/or cationic substituents, nucleation on collagen fibers, is well known [101]. Still, in recent years, several studies [102–105] revealed that HA nanoparticles can be coated by an amorphous, non-apatitic surface layer surrounding a crystalline hydroxyapatite bulk. However, the possibility of obtaining apatitic particles with crystalline structure extended up to the surface was recently reported [106]. In addition, HA surface feature may be modified by exchanging part of calcium ions with other divalent cations. For instance, the presence of Mg<sup>2+</sup> resulted in a significant modification of the surface hydration [107].



Such a behavior is of particular interest because of the widely accepted casual sequence: (i) HA surface structure; (ii) states of adsorbed water; and (iii) states of adsorbed proteins [108]. These latter are the actual “biosurface” that interact with cells during the mineralized tissue healing. Therefore, the possibility to promote the adhesion and proliferation of osteoblasts as well as osteoconductivity *in vivo* on HA coated materials has been explored in a great number of papers. Recently, research seemed to focus particularly on technical optimization of HA coatings. A hybrid nanostructured hydroxyapatite-chitosan composite scaffold was fabricated and successfully tested on human bone marrow stem cells and in a rat model [109]. Mineral substituted hydroxyapatite and carbon nanotube reinforced mineralized hydroxyapatite composite coatings on titanium were prepared by pulsed electrodeposition. An improved viability of osteoblasts was observed *in vitro* on the latter coating, which was also evaluated *in vivo* in Wistar rats [110]. Using a compound surface treatment of sand blasting and acid etching followed by hydroxyapatite radio frequency sputtering, Lai *et al.* assessed the feasibility of a possibly scalable process. [111].

Through Electrophoretic deposition Farnoush *et al.* generated functionally graded coatings of HA/TiO<sub>2</sub> nanoparticles and HA-TiO<sub>2</sub> nanocomposite coatings with different tenors of TiO<sub>2</sub> on Ti-6Al-4V substrate [112]. While thin radio-frequency magnetron sputter deposited nano-hydroxyapatite films were formed on a Fe-tricalcium phosphate bioceramic composite, obtained by conventional powder injection moulding technique [113], amorphous calcium phosphate nanospheres and hydroxyapatite nanorods were prepared and hybridized with polylactic acid to fabricate the composite nanofibers through electrospinning [114].

### 3.2.2. Nanostructured Surface Modifications

A large amount of research has been recently carried out to determine the optimal surface nanotopography for intrabony applications [115]. Titanium dioxide (TiO<sub>2</sub>) nanotubes present numerous advantages as they may be simply obtained by electrochemical anodization or other processes such as the hydrothermal or sol-gel template. In addition, TiO<sub>2</sub> nanotube morphology is correlated with many mesenchymal cell functions such as adhesion, spreading, growth and differentiation, which were shown to be maximally induced on smaller diameter nanotubes (15 nm), but hindered on larger diameter (100 nm) tubes, leading to cell death and apoptosis [115]. Research has supported the significance of nanotopography (TiO<sub>2</sub> nanotube diameter) in cell adhesion and cell growth, and suggests that the mechanics of focal adhesion formation are similar among different cell types. To investigate *in vitro* the effects of surface topography of titanium, a series of micro/nano hierarchical structures were fabricated onto micro-structured titanium substrates via a sol-gel method with spin-coat technique, finding that large grains (80 nm) greatly promoted the proliferation and differentiation of mesenchymal stem cells compared to small grains (20 nm and 40 nm) [116]. In addition, a nanostructured surface was engineered by integrating TiO<sub>2</sub> nanorods into TiO<sub>2</sub> nanodot films. The nanorod integration had an obvious influence on pre-osteoblast cell responses and accelerated cellular osteogenesis. [117]. A thin ribbonlike octacalcium phosphate coating electrodeposited on a hierarchically structured titania surface showed an improved biomineralization ability, in comparison to control, when immersed in simulated body fluid and tested on cells *in vitro* [118]. Modification with a nanoscale TiO<sub>2</sub> coating enhanced the biological response when compared to the uncoated alloy both *in vitro* and *in vivo*. Liu *et al.* [119] evaluated cytocompatibility and early osseointegration of an innovative Ti-24 Nb-4 Zr-7.9 Sn surface. According to Anitua and colleagues [120] based on *in vitro* and *in vivo* analyses, titanium implants modified with calcium ions represented a valuable route to improve osseointegration. Calcium ions, indeed, prevent adventitious hydrocarbon passivation of the oxide layer and produce a durable chemical and nano-topographical modification of the titanium oxide interface.

As biomimetic surface modifications are regarded as promising approach to stimulate cellular behavior at the interface of implant materials [121], plasma based polymerizations methods are acquiring great relevance owing to their efficiency and efficacy. Among them, freshly prepared

amino-terminated films (PPAAm, PPEDA and PSN [59] described in Section 2.3) and deposited on Ti discs, enabled the Human osteoblasts (MG-63 cells, ATCC) to grow in a very flattened and widespread phenotype in the initial phase of adhesion (5 to 30 min). However, a direct correlation between the number of primary amino groups and enhanced cell growth was not found. In fact, not only primary amino groups but also other N-functional groups, such acid amides or imides, play a role for the initial cell functions.

As it may concern the behavior of osteoblasts on the PPEDA obtained by a different plasma process [60] described above (Section 2.3), it was found that the initial cell adhesion after 10 min was significantly increased by the presence of the coating, without any relation to the storage time of up to one year. Although the PPEDA thin film was already chemically modified within 0–30 days after deposition, the cell adhesion was still increased by approximately 55% at day 360. Finally, the additional sterilization process by  $\gamma$ -irradiation has no influence on the improved cell adhesion and proliferation.

The cell viability on another kind of PPAAm coating [61] reported in Section 2.3, deposited on anodically oxidized Ti surfaces and successively derivatized by HyA, was assessed using a direct mitochondrial activity assay (MTT). The cell adhesion (three days after seeding) of the aminated sample (anodized Ti/PPAAm) increased to 67% compared to the untreated Ti sample. This indicates that  $-\text{NH}_2$  groups on the surface can have some positive effects on the implant cytocompatibility. In particular, after six days the anodized Ti/PPAAm/HyA surface showed an 80% increase of cell viability attributed to the improved adhesion and spreading ability of HyA. On the other hand, the viability of MC3T3-E1 cultured on the anodized Ti and anodized Ti/PPAAm samples showed a decrease after six days, confirming that hyaluronic acid is required to enhance cell proliferation.

Representative extracellular matrix proteins fibrinogen, collagen, laminin, and osteopontin were covalently linked to titanium substrates through a PPAAm coating and tested in cell cocultures with interesting results [62]. The biological response shows a distinctly enhanced cell adhesion and cell coverage of HUVECS on fibronectin and collagen modified surfaces at all investigation times compared with the titanium control. For laminin modified surfaces, no cell adhesion is observable as it might be possible that the used HUVECS do not express the suitable integrin receptors that are able to interact with laminin. For HOBS, at all investigation times and on all modified surfaces, cell adhesion and cell coverage was distinctly enhanced compared with the titanium control.

The comparison, as for *in vitro* cell tests, between a PPAAm and a PPAAc film was focused by Schröder and Finke [69]. The positively charged PPAAm film improved, considerably, the spreading of hMSC-, while PPAAc coating reduced the cell area, if compared with bare TiP. Both the films facilitated osteogenic differentiation, which became evident by increased expression of osteogenic differentiation-related mRNA. As for *in vivo* implantation in rats, the PPAAc surfaces caused significantly stronger reactions by macrophages and antigen-presenting cells compared to untreated controls, thus suggesting the PPAAm is more suitable for application of regeneration bone tissue.

In addition, SLA treated Ti surfaces and successively coated by PPAAc, PPAAm and PPAAI films [72] were tested *in vitro* with the MC3T3-E1 cells. PPAAc (COOH)/SLA and PPAAm (NH<sub>2</sub>)/SLA surfaces exhibited more cytoplasmic extensions than on the bare SLA treated Ti and PPAAI(OH)/SLA, with a preference of proliferation for N-containing plasma films, as already previously reported [69].

Biodegradation tests were also performed on Mg discs covered by the same kind of thin plasma polymers [66]. The untreated Mg and PPAAI (OH)/Mg samples exhibited obviously immediately corroded surfaces compared with the other plasma-polymerized samples. In particular, the surface of untreated Mg showed large pits. The plasma polymerized PPAAc (COOH)/Mg and PPAAm(NH<sub>2</sub>)/Mg samples revealed slightly corroded surfaces with only a few small pits and a following better behavior in biodegradation. The plasma-polymerized PPAAc (COOH)/Mg surface was favorable for bonelike apatite formation compared with untreated Mg and plasma-polymerized PPAAm(NH<sub>2</sub>)/Mg and PPAAI(OH)/Mg surfaces.

In addition to nanostructured inorganic substrates, PCL and P<sub>DD</sub>LLA scaffold were coated with the plasma polymers described in [71] and discussed in Section 2.3. After 48 h of Saos2 osteoblasts culture, a higher proliferation of cells on PPE:N/H<sub>2</sub> treated PCL scaffolds with respect to the untreated ones, is observed. On the PPHex/PPAAm coated P<sub>DD</sub>LLA scaffolds, 3T3 fibroblast cell proliferation and a slightly higher metabolic activity after 48 h culture time was observed, with respect to the untreated ones.

Dental implants made of commercially pure titanium are prone to wear and abrasion. Among the surface modifications aiming at improving abrasion resistance, titanium nitride (TiN) coatings were successfully proposed [122]. For the same reason, tantalum is gaining considerable attention for designing a novel type of dental implant, due to its biocompatibility, corrosion resistance and elastic modulus (1.3–10 GPa) close to that of cortical bone (12–18 GPa). Tantalum promotes bone ingrowth by establishing osseoincorporation that will enhance the secondary stability of implants in bone tissue and is more osteoconductive than titanium or cobalt-chromium alloys. [123] Oxidized Ta-based coatings were deposited by reactive DC magnetron sputtering onto Ti CP substrates in an Ar + O<sub>2</sub> atmosphere [124]. Bioactivity tests demonstrated that highest O-content coating showed an increased affinity for apatite adhesion, with higher Ca/P ratio formation, when compared to the bare Ti substrates. Tantalum nitride (TaN) coatings were firstly developed via magnetron sputtering greatly increasing the hardness and modulus of pristine Ti [53]. Antibacterial and corrosion resistance features were described and resulted in better performance in comparison to bare Ti and TiN-coated samples.

An interesting research route aiming at reducing bacterial adhesion not based on drug delivery but rather on the fine modulation of the chemical and topographic characteristics of the interface is worth noting. Designing implants to control bacterial adhesion entails thorough knowledge of their surface. Recurring to magnetron sputtering deposition, amorphous and crystalline titanium oxide and zirconium oxide coatings were produced controlling micro- and nanoscale morphology [41]. The effect of the atomic arrangement or crystallinity was analyzed as for the adhesion of *E. coli* and *S. aureus*. Nano-topography and surface energy were affected by the coating structure: interestingly, at a sub-micro scale, both the crystalline oxides surfaces exhibited higher numbers of attached bacteria. In addition, in a recent study [85], the antibacterial effects of TiO<sub>2</sub> nanotubes could be attributed to the oxidative stress induced as well as geometrical characteristics.

Table 1 provides a synthetic reference on the different types of biological effects that have been reviewed in this section, with proper references.

**Table 1.** Biological effects.

Drug Releasing Surface Modifications	<i>In Vitro</i> Evidence	<i>In Vivo</i> Evidence
Enhancing bone quality	Bisphosphonates [78,79]; BMP-2 [80]; FGF-2 [81]; siRNA nanoparticles [82]	Strontium [36,77]
Antimicrobial features	Silver [86,88]; antibiotic-loaded PHBV microsphere-alginate coatings [90]	Silver [87]; Amikacin and vancomycin [89]
Structural Surface Modifications	<i>In Vitro</i> Evidence	<i>In Vivo</i> Evidence
Hydroxyapatite	nano-HA [97,98]; HA/TiO <sub>2</sub> nanocomposite [112]	Mineral substituted hydroxyapatite and carbon nano tube [110]
Nanostructured surface modifications	TiO <sub>2</sub> nanorods [117]; ribbonlike octacalcium <i>in vitro</i> [118]; amino-terminated films (PPAAm, PPEDA and PSN) [59]; PPEDA [60]; PPAAm [61,62]; PPAAm and PPAAc [69]; PPAAc, PPAAm and PPAAI films [72]; PCL and P <sub>DD</sub> LLA [71]; TiN [122]; porous tantalum [123]	Ti-24 Nb-4 Zr-7.9 Sn [119]; Calcium [120]

#### 4. Conclusions

The most important surface modifications for the improvement of titanium dental implants performance have been reviewed, focusing on the treatments that may develop into novel, sophisticated next generation surfaces that are not only based on tuning roughness and wettability features. Such technologies include inorganic and organic coatings, and will likely be capable of inducing more actively the biological effects desired. Surface modifications have high potential for the improvement of the Ti implant performances, such as the acceleration of osseointegration even in poor quality bone, the protection from chemical corrosion exerted by body fluids, and the reduction of bacterial adhesion. However, further research is required to achieve clinical evidence, at the moment still lacking, supporting the use of the most promising surface modifications.

**Acknowledgments:** This work was partially funded by the Piedmont Region (PAR FSC 2007/13, “Interventi di sostegno per la realizzazione dei poli di innovazione”, project name “FACE”, project number FA 264-204).

**Author Contributions:** Pietro Mandracci contributed to conception, drafted and critically revised the manuscript; Federico Mussano and Paola Rivolo drafted and critically revised the manuscript. Stefano Carossa critically revised the manuscript. All authors gave final approval and agree to accountable for all aspects of the present work.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. Albrektsson, T.; Sennerby, L. State of the art in oral implants. *J. Clin. Periodontol.* **1991**, *18*, 474–481. [[CrossRef](#)] [[PubMed](#)]
2. Schnitman, P.A.; Shulman, L.B., Eds.; Dental implants: Benefit and risk. In Proceedings of An NIH-Harvard Consensus Development Conference, Cambridge, MA, USA, 13–14 June 1978; U.S. Department of Health and Human Services: Bethesda, MD, USA, 1980; pp. 1–351.
3. Zarb, G.A.; Smith, D.C.; Levant, H.C.; Graham, B.S.; Staatsexamen, W.Z. The effects of cemented and uncemented endosseous implants. *J. Prosthet. Dent.* **1979**, *42*, 202–210. [[CrossRef](#)]
4. Cochran, D.L.; Schenk, R.K.; Lussi, A.; Higginbottom, F.L.; Buser, D. Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: A histometric study in the canine mandible. *J. Biomed. Mater. Res.* **1998**, *40*, 1–11. [[CrossRef](#)]
5. Wennerberg, A.; Hallgren, C.; Johansson, C.; Danelli, S. A histomorphometric evaluation of screw-shaped implants each prepared with two surface roughnesses. *Clin. Oral Implants Res.* **1998**, *9*, 11–19. [[CrossRef](#)] [[PubMed](#)]
6. Duraccio, D.; Mussano, F.; Faga, M.G. Biomaterials for dental implants: Current and future trends. *J. Mater. Sci.* **2015**, *50*, 4779–4812. [[CrossRef](#)]
7. Atieh, M.A.; Alsabeeha, N.H.M.; Faggion, C.M., Jr.; Duncan, W.J. The frequency of peri-implant diseases: A systematic review and meta-analysis. *J. Periodontol.* **2013**, *84*, 1586–1598. [[CrossRef](#)] [[PubMed](#)]
8. Uskoković, V. When  $1 + 1 > 2$ : Nanostructured composites for hard tissue engineering applications. *Mater. Sci. Eng. C* **2015**, *57*, 434–451. [[CrossRef](#)] [[PubMed](#)]
9. Schweikl, H.; Müller, R.; Englert, C.; Hiller, K.-A.; Kujat, R.; Nerlich, M.; Schmalz, G. Proliferation of osteoblasts and fibroblasts on model surfaces of varying roughness and surface chemistry. *J. Mater. Sci. Mater. Med.* **2007**, *18*, 1895–1905. [[CrossRef](#)] [[PubMed](#)]
10. Iwaya, Y.; Machigashira, M.; Kanbara, K.; Miyamoto, M.; Noguchi, K.; Izumi, Y.; Ban, S. Surface properties and biocompatibility of acid-etched titanium. *Dent. Mater. J.* **2008**, *27*, 415–421. [[CrossRef](#)] [[PubMed](#)]
11. Yang, G.-L.; He, F.-M.; Yang, X.-F.; Wang, X.-X.; Zhao, S.-F. Bone responses to titanium implants surface-roughened by sandblasted and double etched treatments in a rabbit model. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2008**, *106*, 516–524. [[CrossRef](#)] [[PubMed](#)]
12. Kim, H.; Choi, S.H.; Ryu, J.J.; Koh, S.Y.; Park, J.H.; Lee, I.S. The biocompatibility of SLA-treated titanium implants. *Biomed. Mater.* **2008**, *3*. [[CrossRef](#)] [[PubMed](#)]
13. Park, J.-W.; Jang, I.-S.; Sun, J.-Y. Bone response to endosseous titanium implants surface-modified by blasting and chemical treatment: A histomorphometric study in the rabbit femur. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2008**, *84*, 400–407. [[CrossRef](#)] [[PubMed](#)]

14. Bacchelli, B.; Giavaresi, G.; Franchia, M.; Martinia, D.; de Pasquale, V.; Trirè, A.; Fini, M.; Giardino, R.; Ruggeri, A. Influence of a zirconia sandblasting treated surface on peri-implant bone healing: An experimental study in sheep. *Acta Biomater.* **2009**, *5*, 2246–2257. [[CrossRef](#)] [[PubMed](#)]
15. Guo, C.Y.; Matinlinna, J.P.; Tsoi, J.K.-H.; Tang, H.A.T. Residual contaminations of silicon-based glass, alumina and aluminum grits on a titanium surface after sandblasting. *Silicon* **2015**. In press.
16. Rupp, F.; Scheideler, L.; Olshanska, N.; de Wild, M.; Wieland, M.; Geis-Gerstorfer, J. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. *J. Biomed. Mater. Res. Part A* **2006**, *76A*, 323–334. [[CrossRef](#)] [[PubMed](#)]
17. Le Guéhennec, L.; Soueidan, A.; Layrolle, P.; Amourig, Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent. Mater.* **2007**, *23*, 844–854. [[CrossRef](#)] [[PubMed](#)]
18. Buser, D.; Schenk, R.K.; Steinemann, S.; Fiorellini, J.P.; Fox, C.H.; Stich, H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. *J. Biomed. Mater. Res.* **1991**, *25*, 889–902. [[CrossRef](#)] [[PubMed](#)]
19. Szmukler-Moncler, S.; Perrin, D.; Ahossi, V.; Magnin, G.; Bernard, J.P. Biological properties of acid etched titanium implants: Effect of sandblasting on bone anchorage. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2003**, *68B*, 149–159. [[CrossRef](#)] [[PubMed](#)]
20. Roach, M.D.; Williamson, R.S.; Blakely, I.P.; Didier, L.M. Tuning anatase and rutile phase ratios and nanoscale surface features by anodization processing onto titanium substrate surfaces. *Mater. Sci. Eng. C* **2015**, *58*, 213–223. [[CrossRef](#)] [[PubMed](#)]
21. Wanga, Y.; Yub, H.; Chena, C.; Zhaoa, Z. Review of the biocompatibility of micro-arc oxidation coated titanium alloys. *Mater. Des.* **2015**, *85*, 640–652. [[CrossRef](#)]
22. Gao, Y.-k. Surface modification of TA2 pure titanium by low energy high current pulsed electron beam treatments. *Appl. Surf. Sci.* **2011**, *257*, 7455–7460. [[CrossRef](#)]
23. Surmeneva, M.A.; Surmenev, R.A.; Tyurin, A.I.; Mukhametkaliyev, T.M.; Teresovd, A.D.; Koval, N.N.; Pirozhkova, T.S.; Shuvarin, I.A.; Oehr, C. Comparative study of the radio-frequency magnetron sputter deposited CaP films fabricated onto acid-etched or pulsed electron beam-treated titanium. *Thin Solid Films* **2014**, *571*, 218–224. [[CrossRef](#)]
24. Tian, Y.S.; Chen, C.Z.; Li, S.T.; Huo, Q.H. Research progress on laser surface modification of titanium alloys. *Appl. Surf. Sci.* **2005**, *242*, 177–184. [[CrossRef](#)]
25. Braga, F.J.C.; Marques, R.F.C.; de A. Filho, E.; Guastaldi, A.C. Surface modification of Ti dental implants by Nd:YVO<sub>4</sub> laser irradiation. *Appl. Surf. Sci.* **2007**, *253*, 9203–9208. [[CrossRef](#)]
26. Brånemark, R.; Emanuelsson, L.; Palmquist, A.; Thomsen, P. Bone response to laser-induced micro- and nano-size titanium surface features. *Nanomedicine* **2011**, *7*, 220–227. [[CrossRef](#)] [[PubMed](#)]
27. Györgyey, Á.; Ungvári, K.; Kecskeméti, G.; Kopniczky, J.; Hopp, B.; Oszkó, A.; Pelsöczy, I.; Rakonczay, Z.; Nagy, K.; Turzó, K. Attachment and proliferation of human osteoblast-like cells (MG-63) on laser-ablated titanium implant material. *Mater. Sci. Eng. C* **2013**, *33*, 4251–4259. [[CrossRef](#)] [[PubMed](#)]
28. Cha, S.; Park, Y.-S. Plasma in dentistry. *Clin. Plasma Med.* **2014**, *2*, 4–10. [[CrossRef](#)]
29. Kim, J.-H.; Lee, M.-A.; Han, G.-J.; Cho, B.-H. Plasma in dentistry: A review of basic concepts and applications in dentistry. *Acta Odontol. Scand.* **2014**, *72*, 1–12. [[CrossRef](#)] [[PubMed](#)]
30. Coelho, P.G.; Giro, G.; Teixeira, H.S.; Marin, C.; Witek, L.; Thompson, V.P.; Tovar, N.; Silva, N.R.F.A. Argon-based atmospheric pressure plasma enhances early bone response to rough titanium surfaces. *J. Biomed. Mater. Res. Part A* **2012**, *100A*, 1901–1906. [[CrossRef](#)] [[PubMed](#)]
31. Lee, J.-H.; Kim, Y.-H.; Choi, E.-H.; Kim, K.-M.; Kim, K.-N. Air atmospheric-pressure plasma-jet treatment enhances the attachment of human gingival fibroblasts for early peri-implant soft tissue seals on titanium dental implant abutments. *Acta Odontol. Scand.* **2015**, *73*, 67–75. [[CrossRef](#)] [[PubMed](#)]
32. Yoo, E.-M.; Uhm, S.-H.; Kwon, J.-S.; Choi, H.-S.; Choi, E.H.; Kim, K.-M.; Kim, K.-N. The study on inhibition of planktonic bacterial growth by non-thermal atmospheric pressure plasma jet treated surfaces for dental application. *J. Biomed. Nanotechnol.* **2015**, *11*, 334–341. [[CrossRef](#)] [[PubMed](#)]
33. Yang, C.-H.; Li, Y.-C.; Tsai, W.-F.; Ai, C.-F.; Huang, H.-H. Oxygen plasma immersion ion implantation treatment enhances the human bone marrow mesenchymal stem cells responses to titanium surface for dental implant application. *Clin. Oral Implants Res.* **2015**, *26*, 166–175. [[CrossRef](#)] [[PubMed](#)]



34. Ferraz, E.P.; Sa, J.C.; de Oliveira, P.T.; Alves, C., Jr.; Beloti, M.M.; Rosa, A.L. The effect of plasma-nitrided titanium surfaces on osteoblastic cell adhesion, proliferation, and differentiation. *J. Biomed. Mater. Res. Part A* **2014**, *102A*, 991–998. [[CrossRef](#)] [[PubMed](#)]
35. Liu, C.; Zhang, Y.; Wang, L.; Zhang, X.; Chen, Q.; Wu, B. A strontium-modified titanium surface produced by a new method and its biocompatibility. *PLoS ONE* **2015**, *10*. [[CrossRef](#)] [[PubMed](#)]
36. Wen, J.; Li, J.; Pan, H.; Zhang, W.; Zeng, D.; Xu, L.; Wu, Q.; Zhang, X.; Liu, X.; Jiang, X. Strontium delivery on topographical titanium to enhance bioactivity and osseointegration in osteoporotic rats. *J. Mater. Chem. B* **2015**, *3*, 4790–4804. [[CrossRef](#)]
37. Surmenev, R.A. A review of plasma-assisted methods for calcium phosphate-based coatings fabrication. *Surf. Coat. Technol.* **2012**, *206*, 2035–2056. [[CrossRef](#)]
38. Xie, C.; Lu, H.; Li, W.; Chen, F.-M.; Zhao, Y.-M. The use of calcium phosphate-based biomaterials in implant dentistry. *J. Mater. Sci. Mater. Med.* **2012**, *23*, 853–862. [[CrossRef](#)] [[PubMed](#)]
39. Capuccini, C.; Torricelli, P.; Sima, F.; Boanini, E.; Ristoscu, C.; Bracci, B.; Socol, G.; Fini, M.; Mihailescu, I.N.; Bigi, A. Strontium-substituted hydroxyapatite coatings synthesized by pulsed-laser deposition: *In vitro* osteoblast and osteoclast response. *Acta Biomater.* **2008**, *4*, 1885–1893. [[CrossRef](#)] [[PubMed](#)]
40. Li, Y.; Li, Q.; Zhu, S.; Luo, E.; Li, J.; Feng, G.; Liao, Y.; Hu, J. The effect of strontium-substituted hydroxyapatite coating on implant fixation in ovariectomized rats. *Biomaterials* **2010**, *31*, 9006–9014. [[CrossRef](#)] [[PubMed](#)]
41. Almaguer-Flores, A.; Silva-Bermudez, P.; Galicia, R.; Rodil, S.E. Bacterial adhesion on amorphous and crystalline metal oxide coatings. *Mater. Sci. Eng. C* **2015**, *57*, 88–99. [[CrossRef](#)] [[PubMed](#)]
42. Cimpean, A.; Popescu, S.; Ciofrangeanu, C.M.; Gleizes, A.N. Effects of LP-MOCVD prepared TiO<sub>2</sub> thin films on the *in vitro* behavior of gingival fibroblasts. *Mater. Chem. Phys.* **2011**, *125*, 85–492. [[CrossRef](#)]
43. Battiston, G.A. Dental implants of complex form coated by nanostructured TiO<sub>2</sub> thin films via MOCVD. *Mater. Sci. Forum* **2000**, *352*, 151–158. [[CrossRef](#)]
44. Zhao, X.; Wang, G.; Zheng, H.; Lu, Z.; Zhong, X.; Cheng, X.; Zreiqat, H. Delicate refinement of surface nanotopography by adjusting TiO<sub>2</sub> coating chemical composition for enhanced interfacial biocompatibility. *ACS Appl. Mater. Interfaces* **2013**, *5*, 8203–8209. [[CrossRef](#)] [[PubMed](#)]
45. Song, D.-H.; Uhm, S.-H.; Kim, S.-E.; Kwon, J.-S.; Han, J.-G.; Kim, K.-N. Synthesis of titanium oxide thin films containing antibacterial silver nanoparticles by a reactive magnetron co-sputtering system for application in biomedical implants. *Mater. Res. Bull.* **2012**, *47*, 2994–2998. [[CrossRef](#)]
46. Mandracci, P.; Mussano, F.; Ricciardi, C.; Ceruti, P.; Pirri, F.; Carossa, S. Low temperature growth of thin film coatings for the surface modification of dental prostheses. *Surf. Coat. Technol.* **2008**, *202*, 2477–2481. [[CrossRef](#)]
47. Mandracci, P.; Ceruti, P.; Ricciardi, C.; Mussano, F.; Carossa, S. A-SiO<sub>x</sub> coatings grown on dental materials by PECVD: Compositional analysis and preliminary investigation of biocompatibility improvements. *Chem. Vapor Depos.* **2010**, *16*, 29–34. [[CrossRef](#)]
48. Gazia, R.; Mandracci, P.; Mussano, F.; Carossa, S. AlN<sub>x</sub> and a-SiO<sub>x</sub> coatings with corrosion resistance properties for dental implants. *Surf. Coat. Technol.* **2011**, *206*, 1109–1115. [[CrossRef](#)]
49. Mandracci, P.; Mussano, F.; Ceruti, P.; Pirri, C.F.; Carossa, S. Reduction of bacterial adhesion on dental composite resins by silicon-oxygen thin film coatings. *Biomed. Mater.* **2015**, *10*. [[CrossRef](#)] [[PubMed](#)]
50. Tsai, M.-T.; Chang, Y.-Y.; Huang, H.-L.; Hsu, J.-T.; Chen, Y.-C.; Wu, A.Y.-J. Characterization and antibacterial performance of bioactive Ti-Zn-O coatings deposited on titanium implants. *Thin Solid Films* **2013**, *528*, 143–150. [[CrossRef](#)]
51. Annunziata, M.; Oliva, A.; Basile, M.A.; Giordano, M.; Mazzola, N.; Rizzo, A.; Lanza, A.; Guida, L. The effects of titanium nitride-coating on the topographic and biological features of TPS implant surfaces. *J. Dent.* **2011**, *39*, 720–728. [[CrossRef](#)] [[PubMed](#)]
52. Kim, W.-G.; Choe, H.-C. Effects of TiN coating on the corrosion of nanostructured Ti–30Ta–xZr alloys for dental implants. *Appl. Surf. Sci.* **2012**, *258*, 1929–1934. [[CrossRef](#)]
53. Zhang, Y.; Zheng, Y.; Li, Y.; Wang, L.; Bai, Y.; Zhao, Q.; Xiong, X.; Cheng, Y.; Tang, Z.; Deng, Y.; *et al.* Tantalum nitride-decorated titanium with enhanced resistance to microbiologically induced corrosion and mechanical property for dental application. *PLoS ONE* **2015**, *10*. [[CrossRef](#)] [[PubMed](#)]
54. Wu, H.; Zhang, X.; He, X.; Li, M.; Huang, X.; Hang, R.; Tang, B. Wear and corrosion resistance of anti-bacterial Ti-Cu-N coatings on titanium implants. *Appl. Surf. Sci.* **2014**, *317*, 614–621. [[CrossRef](#)]

55. Huang, H.-L.; Chang, Y.-Y.; Lai, M.-C.; Lin, C.-R.; Lai, C.-H.; Shieh, T.-M. Antibacterial TaN-Ag coatings on titanium dental implants. *Surf. Coat. Technol.* **2010**, *205*, 1636–1641. [[CrossRef](#)]
56. Lai, C.-H.; Chang, Y.-Y.; Huang, H.-L.; Kao, H.-Y. Characterization and antibacterial performance of ZrCN/amorphous carbon coatings deposited on titanium implants. *Thin Solid Films* **2011**, *520*, 1525–1531. [[CrossRef](#)]
57. Liang, Y.; Li, H.; Xu, J.; Li, X.; Qi, M.; Hu, M. Morphology, composition, and bioactivity of strontium-doped brushite coatings deposited on titanium implants via electrochemical deposition. *Int. J. Mol. Sci.* **2014**, *15*, 9952–9962. [[CrossRef](#)] [[PubMed](#)]
58. Abbas, A.; Vivien, C.; Bocquet, B.; Guillochon, D.; Supiot, P. Preparation and multi-characterization of plasma polymerized allylamine films. *Plasma Proc. Polym.* **2009**, *6*, 593–604. [[CrossRef](#)]
59. Finke, B.; Hempel, F.; Testrich, H.; Artemenko, A.; Rebl, H.; Kylián, O.; Meichsner, J.; Biederman, H.; Nebe, B.; Weltmann, K.-D.; *et al.* Plasma processes for cell-adhesive titanium surfaces based on nitrogen-containing coatings. *Surf. Coat. Technol.* **2011**, *205*, S520–S524. [[CrossRef](#)]
60. Testrich, H.; Rebl, H.; Finke, B.; Hempel, F.; Nebe, B.; Meichsner, J. Aging effects of plasma polymerized ethylenediamine (PPEDA) thin films on cell-adhesive implant coatings. *Mater. Sci. Eng. C* **2013**, *33*, 3875–3880. [[CrossRef](#)] [[PubMed](#)]
61. Seo, H.S.; Kim, B.H.; Ko, Y.M. Fabrication of anodized titanium with immobilization of hyaluronic acid to improve biological performance. *Prog. Org. Coat.* **2010**, *69*, 38–44. [[CrossRef](#)]
62. Heller, M.; Kämmerer, P.W.; Al-Nawas, B.; Luszpinski, M.-A.; Förch, R.; Brieger, J. The effect of extracellular matrix proteins on the cellular response of HUVECS and HOBS after covalent immobilization onto titanium. *J. Biomed. Mater. Res. Part A* **2015**, *103A*, 2035–2044. [[CrossRef](#)] [[PubMed](#)]
63. Ricciardi, S.; Castagna, R.; Severino, S.M.; Ferrante, I.; Frascella, F.; Celasco, E.; Mandracci, P.; Vallini, I.; Mantero, G.; Pirri, C.F.; *et al.* Surface functionalization by poly-acrylic acid plasma-polymerized films for microarray DNA diagnostics. *Surf. Coat. Technol.* **2012**, *207*, 389–399. [[CrossRef](#)]
64. Rivolo, P.; Severino, S.M.; Ricciardi, S.; Frascella, F.; Geobaldo, F. Protein immobilization on nanoporous silicon functionalized by RF activated plasma polymerization of acrylic acid. *J. Colloid Interface Sci.* **2014**, *416*, 73–80. [[CrossRef](#)] [[PubMed](#)]
65. Nisticò, R.; Rosellini, A.; Rivolo, P.; Faga, M.G.; Lamberti, R.; Martorana, S.; Castellino, M.; Virga, A.; Mandracci, P.; Malandrino, M.; *et al.* Surface functionalisation of polypropylene hernia-repair meshes by RF-activated plasma polymerisation of acrylic acid and silvernanoparticles. *Appl. Surf. Sci.* **2015**, *328*, 287–295. [[CrossRef](#)]
66. Ko, Y.-M.; Lee, K.; Kim, B.H. Effect of functional groups on biodegradation and pre-osteoblastic cell response on the plasma-polymerized magnesium surface. *Jpn. J. Appl. Phys.* **2013**, *52*. [[CrossRef](#)]
67. Pistillo, B.R.; Perrotta, A.; Gristina, R.; Ceccone, G.; Nardulli, M.; d’Agostino, R.; Favia, P. Water resistant ethylene/acrylic acid plasma-deposited coatings. *Surf. Coat. Technol.* **2011**, *205*, S534–S536. [[CrossRef](#)]
68. Bazaka, K.; Jacob, M.V. Solubility and surface interactions of RF plasma polymerized polyterpenol thin films. *Mater. Express* **2012**, *2*, 285–293.
69. Schröder, K.; Finke, B.; Ohl, A.; Lüthen, F.; Bergemann, C.; Nebe, B.; Rychly, J.; Walschus, U.; Schlosser, M.; Liefelth, K.; *et al.* Capability of differently charged plasma polymer coatings for control of tissue interactions with titanium surfaces. *J. Adhes. Sci. Technol.* **2010**, *24*, 1191–1205. [[CrossRef](#)]
70. Gurzawska, K.; Svava, R.; Yihua, Y.; Haugshøj, K.B.; Dirscherl, K.; Lavery, S.B.; Byg, I.; Damager, I.; Nielsen, M.W.; Jørgensen, B.; *et al.* Osteoblastic response to pectin nanocoating on titanium surfaces. *Mater. Sci. Eng. C* **2014**, *43*, 117–125. [[CrossRef](#)] [[PubMed](#)]
71. Intranuovo, F.; Sardella, E.; Gristina, R.; Nardulli, M.; White, L.; Howard, D.; Shakesheff, K.M.; Alexander, M.R.; Favia, P. PE-CVD processes improve cell affinity of polymer scaffolds for tissue engineering. *Surf. Coat. Technol.* **2011**, *205*, S548–S551. [[CrossRef](#)]
72. Jung, S.-C.; Lee, K.; Kim, B.-H. Biocompatibility of plasma polymerized sandblasted large grit and acid titanium surface. *Thin Solid Films* **2012**, *521*, 150–154. [[CrossRef](#)]
73. Kim, B.H.; Myung, S.W.; Jung, S.C.; Ko, Y.-M. Plasma surface modification for immobilization of bone morphogenic protein-2 on polycaprolactone scaffolds. *Jpn J. Appl. Phys.* **2013**, *52*. [[CrossRef](#)]
74. Da Ponte, G.; Sardella, E.; Fanelli, F.; van Hoeck, A.; d’Agostino, R.; Paulussen, S.; Favia, P. Atmospheric pressure plasma deposition of organic films of biomedical interest. *Surf. Coat. Technol.* **2011**, *205*, S525–S528. [[CrossRef](#)]

75. Bazaka, K.; Jacob, M.V. Synthesis of radio frequency plasma polymerized non-synthetic Terpinen-4-ol thin films. *Mater. Lett.* **2009**, *63*, 1594–1597. [[CrossRef](#)]
76. Bilezikian, J.P.; Raisz, L.G.; Martin, T.J. *Principles of Bone Biology*, 3rd ed.; Academic Press: San Diego, CA, USA, 2008.
77. Li, Y.; Qi, Y.; Gao, Q.; Niu, Q.; Shen, M.; Fu, Q.; Hu, K.; Kong, L. Effects of a micro/nano rough strontium-loaded surface on osseointegration. *Int. J. Nanomed.* **2015**, *10*, 4549–4563.
78. Bosco, R.; Iafisco, M.; Tampieri, A.; Jansen, J.A.; Leeuwenburgh, S.C.G.; van den Beucken, J.J.J.P. Hydroxyapatite nanocrystals functionalized with alendronate as bioactive components for bone implant coatings to decrease osteoclastic activity. *Appl. Surf. Sci.* **2015**, *328*, 516–524. [[CrossRef](#)]
79. Boanini, E.; Torricelli, P.; Sima, F.; Axente, E.; Fini, M.; Mihailescu, I.N.; Bigi, A. Strontium and zoledronate hydroxyapatites graded composite coatings for bone prostheses. *J. Colloid Interface Sci.* **2015**, *448*, 1–7. [[CrossRef](#)] [[PubMed](#)]
80. Wang, Z.; Li, C.; Xu, J.; Wang, K.; Lu, X.; Zhang, H.; Qu, S.; Zhen, G.; Ren, F. Bioadhesive microporous architectures by self-assembling polydopamine microcapsules for biomedical applications. *Chem. Mater.* **2015**, *27*, 848–856. [[CrossRef](#)]
81. Kumorek, M.; Kubies, D.; Filová, E.; Houska, M.; Kasoju, N.; Chánová, E.M.; Matějka, R.; Krýslová, M.; Bačáková, L.; Rypáček, F. Cellular responses modulated by FGF-2 adsorbed on albumin/heparin layer-by-layer assemblies. *PLoS ONE* **2015**, *10*. [[CrossRef](#)] [[PubMed](#)]
82. Song, W.; Song, X.; Yang, C.; Gao, S.; Klausen, L.H.; Zhang, Y.; Dong, M.; Kjems, J. Chitosan/siRNA functionalized titanium surface via a layer-by-layer approach for *in vitro* sustained gene silencing and osteogenic promotion. *Int. J. Nanomed.* **2015**, *10*, 2335–2346.
83. Losic, D.; Aw, M.S.; Santos, A.; Gulati, K.; Bariana, M. Titania nanotube arrays for local drug delivery: Recent advances and perspectives. *Expert Opin. Drug Deliv.* **2015**, *12*, 103–127. [[CrossRef](#)] [[PubMed](#)]
84. Hamlekhan, A.; Sinha-Ray, S.; Takoudis, C.; Mathew, M.T.; Sukotjo, C.; Yarin, A.L.; Shokuhfar, T. Fabrication of drug eluting implants: Study of drug release mechanism from titanium dioxide nanotubes. *J. Phys. D Appl. Phys.* **2015**, *48*. [[CrossRef](#)]
85. Shi, X.; Xu, Q.; Tian, A.; Tian, Y.; Xue, X.; Sun, H.; Yang, H.; Dong, C. Antibacterial activities of TiO<sub>2</sub> nanotubes on *Porphyromonas gingivalis*. *RSC Adv.* **2015**, *5*, 34237–34242. [[CrossRef](#)]
86. Liu, X.-Q.; Chen, Q.-D.; Wang, R.; Wang, L.; Yu, X.-L.; Cao, J.-N.; Zhou, Y.-M.; Sun, H.-B. Simultaneous femtosecond laser doping and surface texturing for implanting applications. *Adv. Mater. Interfaces* **2015**, *2*. [[CrossRef](#)]
87. Qin, H.; Cao, H.; Zhao, Y.; Jin, G.; Cheng, M.; Wang, J.; Jiang, Y.; An, Z.; Zhang, X.; Liu, X. Antimicrobial and osteogenic properties of silver-ion-implanted stainless steel. *ACS Appl. Mater. Interfaces* **2015**, *7*, 10785–10794. [[CrossRef](#)] [[PubMed](#)]
88. Unosson, E.; Rodriguez, D.; Welch, K.; Engqvist, H. Reactive combinatorial synthesis and characterization of a gradient Ag-Ti oxide thin film with antibacterial properties. *Acta Biomater.* **2015**, *11*, 503–510. [[CrossRef](#)] [[PubMed](#)]
89. Jennings, J.A.; Carpenter, D.P.; Troxel, K.S.; Beenken, K.E.; Smeltzer, M.S.; Courtney, H.S.; Haggard, W.O. Novel antibiotic-loaded point-of-care implant coating inhibits biofilm. *Clin. Orthop. Relat. Res.* **2015**, *473*, 2270–2282. [[CrossRef](#)] [[PubMed](#)]
90. Chen, Q.; Li, W.; Goudouri, O.-M.; Ding, Y.; Cabanas-Polo, S.; Boccaccini, A.R. Electrophoretic deposition of antibiotic loaded PHBV microsphere-alginate composite coating with controlled delivery potential. *Colloids Surf. B Biointerfaces* **2015**, *130*, 199–206. [[CrossRef](#)] [[PubMed](#)]
91. Bloemers, F.W.; Blokhuis, T.J.; Patka, P.; Bakker, F.C.; Wippermann, B.W.; Haarman, H.J.Th.M. Autologous bone *versus* calcium-phosphate ceramics in treatment of experimental bone defects. *J. Biomed. Mater. Res. B Appl. Biomater.* **2003**, *66*, 526–531. [[CrossRef](#)] [[PubMed](#)]
92. Zeitler, T.; Cormack, A. Interaction of water with bioactive glass surfaces. *J. Cryst. Growth* **2006**, *294*, 96–102. [[CrossRef](#)]
93. Pereira, M.; Jones, J.; Hench, L. Bioactive glass and hybrid scaffolds prepared by sol-gel. *Adv. Appl. Ceram.* **2005**, *104*, 35–42. [[CrossRef](#)]
94. LeGeros, R. Calcium phosphate-based osteoinductive materials. *Chem. Rev.* **2008**, *108*, 4742–4753. [[CrossRef](#)] [[PubMed](#)]

95. Kandori, K.; Fudo, A.; Ishikawa, T. Surface structure and properties of fluoridated calcium hydroxyapatite. *Colloids Surf. B* **2002**, *24*, 145–149. [[CrossRef](#)]
96. Robinson, R.A. An electron microscope study of the crystalline inorganic components of bone and its relationship to the organic matrix. *J. Bone Joint Surg.* **1952**, *34A*, 389–434. [[PubMed](#)]
97. Webster, T.J.; Siegel, R.; Bizios, R. Osteoblast adhesion on nanophase ceramics. *Biomaterials* **1999**, *20*, 1221–1227. [[CrossRef](#)]
98. Webster, T.J.; Ergun, C.; Doremus, R.H.; Siegel, R.W.; Bizios, R. Enhanced functions of osteoblasts on nanophase ceramics. *Biomaterials* **2000**, *21*, 1803–1810. [[CrossRef](#)]
99. Rinnerthaler, S.; Roschger, P.; Jakob, F.; Nader, K.; Klaushofer, P.F. Scanning small angle X-ray scattering analysis of human bone sections. *Calcif. Tissue Int.* **1999**, *64*, 422–426. [[CrossRef](#)] [[PubMed](#)]
100. Žižak, I.; Paris, O.; Roschger, P.; Bernstorff, S.; Amenitsch, H.; Klaushofer, K.; Fratzl, P. Investigation of bone and cartilage by synchrotron scanning-SAXS and -WAXD with micrometer spatial resolution. *J. Appl. Cryst.* **2000**, *33*, 820–823.
101. Celotti, G.; Tampieri, A.; Sprio, S.; Landi, E.; Bertinetti, L.; Martra, G.; Ducati, C. Crystallinity in apatites: How can a truly disordered fraction be distinguished from nanosize crystalline domains? *J. Mater. Sci. Mater. Med.* **2006**, *17*, 1079–1087. [[CrossRef](#)] [[PubMed](#)]
102. Isobe, T.; Nakamura, S.; Nemoto, R.; Senna, M. Solid-state double nuclear magnetic resonance study of the local structure of calcium phosphate nanoparticles synthesized by a wet-mechanochemical reaction. *J. Phys. Chem. B* **2002**, *106*, 5169–5176. [[CrossRef](#)]
103. Cazalbou, S.; Combes, C.; Eichert, D.; Rey, C. Adaptive physico-chemistry of bio-related calcium phosphates. *J. Mater. Chem.* **2004**, *14*, 2148–2153. [[CrossRef](#)]
104. Jaeger, C.; Welzel, T.; Meyer-Zaika, W.; Epple, M. A solid-state NMR investigation of the structure of nanocrystalline hydroxyapatite. *Magn. Reson. Chem.* **2006**, *44*, 573–580. [[CrossRef](#)] [[PubMed](#)]
105. Bertinetti, L.; Tampieri, A.; Landi, E.; Ducati, C.; Midgley, P.A.; Coluccia, S.; Martra, G. Surface structure, hydration, and cationic sites of nanohydroxyapatite: UHR-TEM, IR, and microgravimetric studies. *J. Phys. Chem. C* **2007**, *111*, 4027–4035. [[CrossRef](#)]
106. Sakhno, Y.; Bertinetti, L.; Iafisco, M.; Tanmieri, A.; Roveri, N.; Martra, G. Surface hydration and cationic sites of nanohydroxyapatites with amorphous or crystalline surfaces: A comparative study. *J. Phys. Chem. C* **2010**, *114*, 16640–16648. [[CrossRef](#)]
107. Bertinetti, L.; Drouet, C.; Combes, C.; Rey, C.; Tampieri, A.; Coluccia, S.; Martra, G. Surface characteristics of nanocrystalline apatites: Effect of Mg surface enrichment on morphology, surface hydration species, and cationic environments. *Langmuir* **2009**, *25*, 5647–5654. [[CrossRef](#)] [[PubMed](#)]
108. Kasemo, B. Biological surface science. *Surf. Sci.* **2002**, *500*, 656–677. [[CrossRef](#)]
109. Guo, Y.-P.; Guan, J.-J.; Yang, J.; Wang, Y.; Zhang, C.-Q.; Ke, Q.-F. Hybrid nanostructured hydroxyapatite-chitosan composite scaffold: Bioinspired fabrication, mechanical properties and biological properties. *J. Mater. Chem. B* **2015**, *3*, 4679–4689. [[CrossRef](#)]
110. Gopi, D.; Shinyjoy, E.; Karthika, A.; Nithiya, S.; Kavitha, L.; Rajeswari, D.; Tang, T. Single walled carbon nanotubes reinforced mineralized hydroxyapatite composite coatings on titanium for improved biocompatible implant applications. *RSC Adv.* **2015**, *5*, 36766–36778. [[CrossRef](#)]
111. Lai, H.-C.; Tsai, H.-H.; Hung, K.-Y.; Feng, H.-P. Fabrication of hydroxyapatite targets in radio frequency sputtering for surface modification of titanium dental implants. *J. Int. Mater. Sys. Struct.* **2015**, *26*, 1050–1058. [[CrossRef](#)]
112. Farnoush, H.; Aldiç, G.; Çimenoglu, H. Functionally graded HA-TiO<sub>2</sub> nanostructured composite coating on Ti-6Al-4V substrate via electrophoretic deposition. *Surf. Coat. Technol.* **2015**, *265*, 7–15. [[CrossRef](#)]
113. Surmeneva, M.A.; Kleinhans, C.; Vacun, G.; Kluger, P.J.; Schönhaar, V.; Müller, M.; Hein, S.B.; Wittmar, A.; Ulbricht, M.; Prymak, O.; et al. Nano-hydroxyapatite-coated metal-ceramic composite of iron-tricalcium phosphate: Improving the surface wettability, adhesion and proliferation of mesenchymal stem cells *in vitro*. *Colloids Surf. B Biointerfaces* **2015**, *135*, 386–393. [[CrossRef](#)] [[PubMed](#)]
114. Zhang, H.; Fu, Q.-W.; Sun, T.-W.; Chen, F.; Qi, C.; Wu, J.; Cai, Z.-Y.; Qian, Q.-R.; Zhu, Y.-J. Amorphous calcium phosphate, hydroxyapatite and poly(D,L-lactic acid) composite nanofibers: Electrospinning preparation, mineralization and *in vivo* bone defect repair. *Colloids Surf. B Biointerfaces* **2015**, *136*, 27–36. [[CrossRef](#)] [[PubMed](#)]

115. Kulkarni, M.; Mazare, A.; Gongadze, E.; Perutkova, Š.; Kralj-Iglic, V.; Milošev, I.; Schmuki, P.; Iglič, A.; Mozetič, M. Titanium nanostructures for biomedical applications. *Nanotechnology* **2015**, *26*. [[CrossRef](#)] [[PubMed](#)]
116. Shen, X.; Ma, P.; Hu, Y.; Xu, G.; Zhou, J.; Cai, K. Mesenchymal stem cell growth behavior on micro/nano hierarchical surfaces of titanium substrates. *Colloids Surf. B Biointerfaces* **2015**, *127*, 221–232. [[CrossRef](#)] [[PubMed](#)]
117. Cheng, K.; Yu, M.; Liu, Y.; Ge, F.; Lin, J.; Weng, W.; Wang, H. Influence of integration of TiO<sub>2</sub> nanorods into its nanodot films on pre-osteoblast cell responses. *Colloids Surf. B Biointerfaces* **2015**, *126*, 387–393. [[CrossRef](#)] [[PubMed](#)]
118. Jiang, P.; Liang, J.; Song, R.; Zhang, Y.; Ren, L.; Zhang, L.; Tang, P.; Lin, C. Effect of octacalcium-phosphate-modified micro/nanostructured titania surfaces on osteoblast response. *ACS Appl. Mater. Interfaces* **2015**, *7*, 14384–14396. [[CrossRef](#)] [[PubMed](#)]
119. Liu, X.H.; Wu, L.; Ai, H.J.; Han, Y.; Hu, Y. Cytocompatibility and early osseointegration of nanoTiO<sub>2</sub>-modified Ti-24 Nb-4 Zr-7.9 Sn surfaces. *Mater. Sci. Eng. C* **2015**, *48*, 256–262. [[CrossRef](#)] [[PubMed](#)]
120. Anitua, E.; Piñas, L.; Murias, A.; Prado, R.; Tejero, R. Effects of calcium ions on titanium surfaces for bone regeneration. *Colloids Surf. B Biointerfaces* **2015**, *130*, 173–181. [[CrossRef](#)] [[PubMed](#)]
121. Vallée, A.; Faga, M.G.; Mussano, F.; Catalano, F.; Tolosano, E.; Carossa, S.; Altruda, F.; Martra, G. Alumina-zirconia composites functionalized with laminin-1 and laminin-5 for dentistry: Effect of protein adsorption on cellular response. *Colloids Surf. B Biointerfaces* **2014**, *114*, 284–293. [[CrossRef](#)] [[PubMed](#)]
122. Shi, X.; Xu, L.; Munar, M.L.; Ishikawa, K. Hydrothermal treatment for TiN as abrasion resistant dental implant coating and its fibroblast response. *Mater. Sci. Eng. C* **2015**, *49*, 1–6. [[CrossRef](#)] [[PubMed](#)]
123. Liu, Y.; Bao, C.; Wismeijer, D.; Wu, G. The physicochemical/biological properties of porous tantalum and the potential surface modification techniques to improve its clinical application in dental implantology. *Mater. Sci. Eng. C* **2015**, *49*, 323–329. [[CrossRef](#)] [[PubMed](#)]
124. Almeida Alves, C.F.; Cavaleiro, A.; Carvalho, S. Bioactivity response of Ta<sub>1-x</sub>O<sub>x</sub> coatings deposited by reactive DC magnetron sputtering. *Mater. Sci. Eng. C* **2016**, *58*, 110–118. [[CrossRef](#)] [[PubMed](#)]



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).